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REVIEW ARTICLE

Role of Brain NUCB2/nesfatin-1 in the Stress-induced Modulation of Gastrointestinal Functions

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Abstract: ***Background***: Nucleobindin2 (NUCB2)/nesfatin-1 plays a well-established role in homeostatic functions associated with food intake and stress integration.

Aim: This review focusses on NUCB2/nesfatin-1's central effects on gastrointestinal functions and will summarize the effects on food intake, motility and secretion with focus on the upper gastrointestinal tract.

Results: We will highlight the stressors that influence brain NUCB2/nesfatin-1 expression and discuss functional implications. In addition to traditional acute psychological and physical stressors such as restraint stress and abdominal surgery we will look at immunological, visceral and metabolic stressors as well as a chronic combination stress model that have been shown to affect NUCB2/nesfatin-1 signaling and describe associated functional consequences.

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Keywords: Brain-gut axis, corticotropin releasing factor, CRF, food intake, motility, secretion.

INTRODUCTION

The gastrointestinal tract, an organ system very susceptible to stress [1], can be divided into an upper and lower part comprised of oesophagus, stomach and upper small bowel in the upper part and lower small bowel and colon in the lower part. Similar to the corticotropin releasing factor (CRF), a hallmark transmitter of the stress response [2], nucleobindin2 (NUCB2) and its cleavage product nesfatin-1 [3] affect the functions of the upper and lower gastrointestinal tract. An interaction of NUCB2 and CRF has been postulated before. Central injection of nesfatin-1 activates CRF-containing brain regions such as the parvicellular part of the paraventricular nucleus of the hypothalamus (PVN) [4] and leads to release of peripheral adreno-corticotropic hormone (ACTH) and corticosterone [4, 5]. More evidence for a direct interaction of nesfatin-1 with hypothalamic CRF neurons comes from experiments by Yoshida *et al*. who showed elevated calcium influx in CRF neurons after stimulation with nesfatin-1 *in vitro* [5]. Furthermore, the anorexigenic effects of NUCB2/nesfatin-1 were blocked by simultaneous application of both, an unspecific CRF antagonist as well as a specific CRF_2-antagonist, astressin$_2$-B which highlights the possible recruitment and interaction of NUCB2/nesfatin-1 with the CRF system [6].

These data give rise to an involvement of NUCB2/nesfatin-1 in the response to stress. The present review will describe the effects of NUCB2/nesfatin-1 on food intake and gastrointestinal functions and the associated modulation under conditions of a variety of different stressors.

EFFECTS OF CENTRAL NUCB2/NESFATIN-1 ON GASTROINTESTINAL FUNCTIONS

Food Intake

The first report by Oh-I *et al*. showed that intracerebroventricular (icv) injection of nesfatin-1 but not the other fragments cleaved from NUCB2, nesfatin-2 or nesfatin-3, exerted an anorexigenic effect in rats [3]. Over the years, other groups confirmed the nocturnal anorexigenic effects of nesfatin-1 in rats after central injection into the 2nd, 3rd or 4th brain ventricle or into the cisterna magna [6-10]. Nesfatin-1 also affects body weight as shown by a decrease observed at 24 h post injection [6], a finding most likely related to an increase in energy expenditure [11]. In line with these data, chronic central infusion of an anti nesfatin-1 antisense oligonucleotide (as-MON) increased food intake and body weight [3]. This gives rise to a physiological role of NUCB2/nesfatin-1 in the regulation of food intake.

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Central injection of nesfatin-1 in mice (2^{nd} and 3^{rd} brain ventricle) also decreases food intake in a dose-dependent manner [8, 12-14]. The anorexigenic effect of full length nesfatin-1 is comprised of a reduction in meal size (indicating increased satiation) and meal frequency (as an indicator for increased satiety) [13] in mice and can be attributed to the active core of nesfatin-1, nesfatin1_{30-59}, in mice [14] and rats [15]. Interestingly, the food intake microstructure observed following the injection of nesfatin-1_{30-59} differed with an increase of satiety in mice [14], while in rats satiation was induced [15]. Despite the growing body of evidence on the role of nesfatin-1 as anorexigenic modulator in rodents, the confirmation of these effects in humans is still pending.

The peripheral effects of NUCB2/nesfatin-1 on feeding are reviewed elsewhere [16]. Quickly summarized, in mice, peripheral injection of nesfatin-1 resulted in differing effects: In one study nesfatin-1 injected intraperitoneally (ip) decreased food intake [17] and activated neurons in the nucleus of the solitary tract (NTS) and area postrema (AP). However, another report was unable to observe a decrease in food intake when nesfatin-1 was injected ip or subcutaneously (sc) in mice at a 23-times higher dose compared to central injection [13]. Therefore, the effect of peripheral nesfatin-1 on food intake has still to be established yet. However, there is converging evidence that peripheral nesfatin-1 exerts effects on upper gastrointestinal motility and secretion.

Effect of Fasting and Re-feeding

Conditions of negative energy balance, such as 48-h fasting or sustained subnutrition, induce a decrease in hypothalamic NUCB2 mRNA and/or protein levels in pubertal female rats [18]. In line with these data, fasting for 24 h reduced plasma levels of NUCB2/nesfatin-1 compared with *ad libitum* fed rats [6]. However, there was no statistically significant difference between fasting and postprandial nesfatin-1 levels in obese children [19]. Whether this reflects a lack of translation from animals to humans or is due to other confounding factors will have to be further investigated.

Re-feeding after a 48-h fast results in a 10-fold increase of activated NUCB2/nesfatin-1 neurons in the PVN and a 30-fold increase in the supraoptic nucleus (SON) as assessed by Fos immunoreactivity (IR) [20]. Also, the total amount of nesfatin-1 IR and expression of NUCB2 mRNA in the SON was significantly higher compared to fasted rats [20] indicating that hypothalamic nesfatin-1 expression can be modulated by feeding.

Hypothalamic NUCB2 mRNA expression is responsive to leptin as treatment with leptin *in vivo* and *in vitro* markedly increased NUCB2 expression in the PVN [21]. NUCB2/nesfatin-1 is likely to act downstream of leptin to modulate food intake based on the finding that leptin failed to decrease food intake in mice with hypothalamic shRNA-silenced NUCB2 gene expression [21]. The same group further demonstrated that paraventricular NUCB2/nesfatin-1 neurons are activated as shown by increased calcium influx upon glucose and insulin superfusion as well as leptin treatment [21, 22]. Thus, meal-evoked activation of PVN NUCB2/nesfatin-1 neurons could be an important factor in the induction of satiation and satiety [22].

Besides the meal-dependent regulation of NUCB2/nesfatin-1, hypothalamic paraventricular NUCB2 mRNA expression also underlies a circadian variation within increase during the early light phase [23], the phase were rats show their minimum food intake [24]. Further supporting the assumption of an important role of NUCB2/nesfatin-1 in the light-phase associated reduction of food intake, treatment with an anti-nesfatin-1 IgG to immuno-neutralize NUCB2/nesfatin-1 in the PVN increased food intake in the light but not during the dark phase [23]. Whether this circadian regulation of NUCB2/nesfatin-1 is also observable in humans and exerts similar functions has to be subject of future studies.

Gastric Motility and Secretion

Central injection of nesfatin-1 (3 μg/mouse) in 8-h food deprived mice at the beginning of the dark phase resulted in a significant reduction of gastric emptying assessed during the 150–170 min period after icv injection [25], a finding that corresponds to the maximal response of the dark phase food intake suppression [6, 8, 13]. Similarly, central (lateral brain ventricle) nesfatin-1 induced a dose-dependent reduction of gastric emptying that was not CRF_2-dependent in rats indicating a dissociation from the anorexigenic effect [6]. Furthermore, central injection of nesfatin-1 into the lateral brain ventricle inhibited antroduodenal motility in a dose-dependent manner in mice [12]. This effect is most likely mediated by the hypothalamus as recent studies showed that direct administration of nesfatin-1 into the arcuate nucleus (Arc) or PVN also decreased gastric motility [26, 27], which was partly prevented by an oxytocin receptor antagonist [26] or a melanocortin$_{3/4}$ receptor antagonist [28] and increased by pre-administration of an anti-NUCB2/nesfatin-1 antibody while lateral hypothalamic area (LHA) neurons had a modulatory function [26]. Another group showed that nesfatin-1 injection into the central amygdala in rats reduces gastric motility, an effect prevented by subdiaphragmatic vagotomy [28]. However, fourth ventricular injection of nesfatin-1 did not have an effect on gastric emptying [6]. Thus, there is different regulation of gastric functions depending on the brain area involved and forebrain systems seem to be more important than hindbrain systems in the mediation of nesfatin-1's gastric motility reducing effects. Taken together, hypothalamic and amygdaloid (forebrain) neurons are involved in the nesfatin-1 induced modulation of gastric functions and dependent on vagal stimulation as well as melanocortin and oxytocin signalling.

In addition, also peripheral (intravenous, iv) injection of nesfatin-1 reduced gastric contractions and inhibited cyclical interdigestive migrating contractions in fasted Beagle dogs [29]. There is also evidence that ip (5-40 μg/kg) and icv (5-1000 ng/rat) nesfatin-1 decreases gastric acid secretion in rats in a dose dependent manner [30]. Thus, gastric mucosal damage induced with the combined stressor water immersion and restraint or indomethacin injection was attenuated by ip nesfatin-1 by increasing gastric mucosal blood flow and

decreasing gastric acid secretion [30, 31]. From their results, Szlachzic *et al*. and Kolgazi *et al*. conclude that nesfatin-1 has gastroprotective effects towards stress-associated mucosal damage through activation of cyclooxygenase and prostaglandins, the oxidant/antioxidant system, vanilloid receptors and sensory nerves releasing vasodilatory mediators such as calcitonin-gene related peptide (CGRP) [30, 31]. Further protective mechanisms may involve a decreased expression of proinflammatory cytokines such as interleukin (IL)-6, IL-1b and tumor necrosis factor (TNF)-α [30-32]. A role for the vagus nerve was further postulated by Xia *et al*. who showed that fourth ventricular nesfatin-1 did not affect basal and pentagastrin-stimulated gastric acid secretion but inhibited vagally stimulated gastric acid production in a dose-dependent manner in rats [33].

Colonic Motility, Secretion and Defecation

Although immunohistochemical staining showed that NUCB2/nesfatin-1 immunoreactive (ir) cells are distributed throughout the gastrointestinal tract with localization in endocrine cells of the gastric mucosa (co-localized with ghrelin) [6], duodenal Brunner's glands, small intestine and colon in Sprague-Dawley rats, ICR mice and dogs [34, 35] there have been no studies so far on the effects of nesfatin-1 on colonic motility, secretion and defecation.

Visceral Hypersensitivity

Visceral hypersensitivity is a condition often found in irritable bowel syndrome (IBS). In rats, IBS-like visceral hypersensitivity was induced by intracolonic infusion of 0.5% acetic acid at an early age [36]. Serum nesfatin-1 levels that were measured in response to colorectal distension were significantly higher in the hypersensitive rats compared to controls [36]. Upon icv injection of an anti-nesfatin-1/NUCB2 antibody or a selective CRF_1-antagonist, rats responded less hypersensitive to colorectal distension which shows an implication of NUCB2/nesfatin-1 and the central CRF_1-pathway in mediation of visceral hypersensitivity [36].

STRESS MODELS THAT ACTIVATE BRAIN NUCB2/ NESFATIN-1 AND FUNCTIONAL IMPLICATIONS

NUCB2/nesfatin-1's distribution in stress responsive brain neuronal circuitries including hypothalamic, limbic and hindbrain systems supports a role for this peptide in the stress response. Double labelling with Fos protein, a product of the immediate early gene c-Fos is a widely used model to assess acute neuronal activation. Fos immunohistochemistry has been detected specifically in NUCB2/nesfatin-1 containing brain nuclei that are activated by various physical and psychological stressors. On the other hand, icv injection of nesfatin-1 elevated ACTH and corticosterone levels that peaked after 10-30 and 15-60 min, respectively [4, 5] and bilateral adrenalectomy elicited increased NUCB2 mRNA expression in the PVN, indicating a negative feedback mechanism in rats [4]. Furthermore, icv injected nesfatin-1 induces robust Fos expression in hypothalamic and brainstem nuclei associated with the stress response such as the PVN, SON, locus coeruleus (LC), raphe nuclei and NTS [5] pointing towards the expression of the (yet unknown) nesfatin-1 receptor in these brain nuclei. Further evidence for this hypothesis was recently provided by an autoradiography study showing strong ^{125}I-nesfatin-1 signals in the PVN and NTS [37].

The central expression of NUCB2/nesfatin-1 in feeding regulatory brain centers and their co-localization with neuropeptides such as neuropeptide Y (NPY), proopiomelanocortin (POMC) and cocaine- and amphetamine regulated transcript (CART), oxytocin and vasopressin and CRF as well as detailed mapping of brain regions activated by central injection of nesfatin-1 have been thoroughly described elsewhere [38-41]. In the present review we will focus on regions implicated in the nesfatin-1 modulated response to stress. The following paragraphs will give an overview on the stress models that have been explored with regards to central activation of NUCB2/nesfatin-1 (central mediation of NUCB2/nesfatin-1 signalling is summarized in Fig. **1**; details on the stress models are provided in Table **1**).

Psychological Stress

Restraint Stress

Restraint stress is a combined physical-psychological stressor that forces rats in an immobile position over a defined period of time, usually varying between minutes to hours. This stress paradigm was shown to activate NUCB2/ nesfatin-1 immunoreactive neurons in the hypothalamus and hindbrain as shown by Fos immunohistochemistry [4, 5, 42] and elevated NUCB2 mRNA expression in the PVN and ventrolateral medulla (VLM) [4]. Specifically, restraint induced significant Fos expression in neurons of the SON, PVN, LC, rostral raphe pallidus (rRPa), NTS, and VLM [5, 42]. Double Fos/nesfatin-1 labelling indicated that Fos-ir neurons comprised the majority of nesfatin-1-ir cells in the SON, VLM, rRPA and LC, and ~ 50% in the caudal NTS and anterior parvicellular PVN. The localization of restraint activated neurons in the PVN overlaps with CRF-containing neurons [43-45]. Restraint stress failed to elevate NUCB2/ nesfatin-1 plasma levels in venous or portal vein blood [5] giving rise to a central mode of action.

In the non-preganglionic (np) Edinger-Westphal nucleus (EW) of mice, restraint stress stimulated the production of nesfatin-1 as shown by a 2.6-times increase of mRNA production compared to controls pointing towards a specific role of NUCB2/nesfatin-1 in the response to acute stress [46]. However, another study failed to detect npEW mRNA changes of NUCB2 following acute or chronic stress [47] but described increased activation of nesfatin-1 immunoreactive neurons of the npEW [47] as well as an increase in nesfatin-1 IR.

Centrally projecting neurons of the Edinger Westphal nucleus (cpEW) have been implicated in the development of stress-related mood disorders like major depression in a sex-specific manner. Bloem *et al*. described the NUCB2 mRNA content in the cpEW to be higher in male compared to female suicide victims, where they were lower compared to controls [48]. In another collective of anxiety disorder patients, plasma nesfatin-1 levels were found to be significantly lower compared to controls [49]. In contrast, under conditions of perceived anxiety, stress and depression in obese women plasma NUCB2/nesfatin-1 levels were

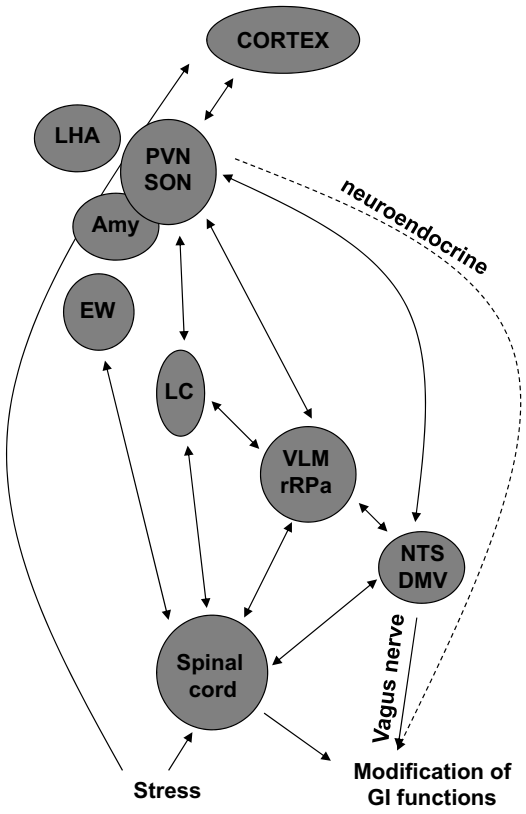

Fig. (1). Possible route of central mediation of stress induced signals by NUCB2/nesfatin-1. The arrow indicates the direction of signalling between nuclei/areas involved in the mediation of stress that all express NUCB2/nesfatin-1. NUCB2/nesfatin-1 has been mainly detected in neuronal cell bodies, while no or less expression was observed in nerve terminals. Therefore, it is unclear whether it acts as a classical neurotransmitter. However, since dendrites and soma can also secrete transmitters [72, 73], NUCB2/nesfatin-1 could act in an extracellular manner. Alternatively and/or additionally, direct NUCB2/nesfatin-1 connections as recently shown between the dorsal vagal complex and the paraventricular nucleus [74] may play a role. Lastly, the dotted line indicates a possible direct endocrine mediation pathway of NUCB2/nesfatin-1 by crossing the blood-brain barrier which has been observed before [75, 76]. Abbreviations: Amy, amygdala; DMV, dorsal motor nucleus of the vagus nerve; EW, Edinger-Westphal nucleus; LHA, lateral hypothalamic area; LC, locus coeruleus; NTS, nucleus of the solitary tract; PVN, paraventricular nucleus; rRPa, rostral raphe pallidus; SON, supraoptic nucleus; VLM, ventrolateral medulla.

elevated in subjects with higher anxiety compared to those that reported less anxiety [50]. These data point towards a sex specific regulation of nesfatin-1, a hypothesis supported by a recent study [51]. Lastly, whether these associations represent causal relationships will have to be further established as well – animal data point towards an induction of anxiety following central injection of nesfatin-1 [52, 53].

Water Avoidance Stress

Water avoidance stress (WAS), a psychological stressor where the animals stand on a small platform to avoid water contact, in mice activated several brain nuclei implicated in the stress response such as the PVN, LC, rRPa, VLM and NTS where 35%, 50%, 54%, 58% and ~35%, respectively, of neurons were nesfatin-1-ir [25] suggesting a role of nesfatin-1 in the response to this stressor. Also hypothalamic NUCB2 mRNA expression was increased by WAS which may contribute to the elevated NUCB2/nesfatin-1 plasma levels observed under these conditions [54].

Physical Stress

Abdominal Surgery and Postoperative Ileus

Postoperative ileus is a clinical condition that usually develops after abdominal surgery in humans. It is characterized by delayed gastric emptying and prolongation of intestinal transit [55]. Clinical symptoms encompass absent bowel movements, flatus and defecation associated with abdominal bloating and pain. The ileus is defined as time from the surgery until passage of flatus or stool together with the time to adequate oral nutrient intake [56].

In animal research, abdominal surgery can be used as a model for physical stress and pain. To mimic abdominal surgery, rodents receive a median laparotomy under general anesthesia followed by short cecal palpation, usually of 1-min duration [57, 58]. Recent studies indicated that icv nesfatin-1 delayed gastric emptying in rats [6] and reduced gastroduodenal motility in mice [12] thus demonstrating a role for nesfatin-1 in upper gastrointestinal motility. Abdominal surgery induces a robust activation of NUCB2/nesfatin-1-immunoreactive neurons in the magnocellular neuroendocrine system, in the anterior parvicellular part of the PVN, EW, and nuclei of the catecholaminergic and serotonergic system [59]. Taken together, under these conditions activated NUCB2/nesfatin-1 signalling after abdominal surgery may contribute to the decrease of food intake and gastrointestinal transit. This has to be further investigated in future studies.

Visceral Stress

Gastric Distension

Gastric or colonic distension are considered visceral stressors. Bonnet *et al*. described NUCB2/nesfatin-1 immunoreactive neurons to be activated in the NTS after gastric distension in rats. Moreover, retrogradely fluorogold labelled NUCB2/nesfatin-1 neurons corresponding to gastric vagal efferent preganglionic neurons were detected in the DMN and arose from the stomach level [60]. Taken together, hindbrain NUCB2/nesfatin-1 may be involved in the mediation of the response to visceral stress.

Immunological Stress

Injection of Lipopolysaccharide (LPS)

Systemic administration of the gram-negative bacterial wall component lipopolysaccharide (LPS) is a well-established model for immunological stress. Peripheral injection of LPS activates NUCB2/nesfatin-1-immunopositive neurons in hypothalamic and hindbrain nuclei such as the PVN, SON, Arc and NTS [61] and increases plasma NUCB2/nesfatin-1 concentrations for up to 7 hours post injection [62]. The increase at 2 h was paralleled by an increase in gastric corpus NUCB2 mRNA and protein

Table 1. Effect of different stressors in NUCB2/nesfatin-1 signaling in experimental animals.

Stressor	Details of Stress Paradigm and Duration	Effects	Method	Refs.
Psychological stress				
Restraint stress	15 min wrap restraint in wire mesh	no effect on nesfatin-1 levels in venous or portal blood	radioimmunoassay rats	[5]
Restraint stress	30 min wrap restraint in plastic bags	Fos induction in nesfatin-1-ir neurons of SON, apPVN, lmPVN, mmPVN, mpPVN, LC, rRPa, VLM, cNTS no Fos induction in: ventral BNST, septum, cingulate, piriform, and entorhinal cortex, amygdaloid nuclei, DMN	Fos immunohistochemistry whole brain mapping rats	[42]
Restraint stress	1 h wrap restraint in wire mesh	Fos induction in nesfatin-1-ir neurons of SON, PVN, Arc, NTS and LC	Fos immunohistochemistry rats	[5]
Restraint stress	1 h restraint in transparent plastic tubes	Fos induction in CART/nesfatin-1-ir neurons of npEW no effect on nesfatin-1-ir immunoreactivity in npEW	immunohistochemistry rats	[47]
Restraint stress	2 h restraint in transparent plastic tubes	elevation of NUCB2 mRNA in npEW	q-RT-PCR mice	[46]
Restraint stress	4 h restraint in transparent plastic tubes	Fos induction in mpPVN	immunohistochemistry rats	[4]
Restraint stress	4 h restraint in transparent plastic tubes	NUCB2/nesfatin-1 mRNA in mpPVN and VLM	in situ hybridization rats	[4]
Water avoidance stress	mice on a rectangular platform located inside a container filled with warm water up to 1 cm below the top of the platform for 1 h	Fos induction in nesfatin-1-ir neurons of PVN, LC, rRPa, VLM, mNTS and cNTS no increase in EW and rNTS	immunohistochemistry ad libitum fed mice	[25]
Physical stress				
Abdominal surgery	sterile surgery with median laparotomy and 1 min cecal palpation under general isoflurane anesthesia	Fos induction in nesfatin-1-ir neurons of SON, apPVN, lmPVN, mmPVN, mpPVN, LC, rRPa, EW, VLM and NTS	immunohistochemistry rats	[59]
Gastric distension	isobaric distension of a spherical shaped plastic balloon with a non-compliant catheter inserted to the proximal stomach through an incision at the tip of the fundus isobaric phasic distensions to a constant pressure of 60mmHg for 20-s with a 4-min inter-stimulus interval for 2h	Fos induction in nesfatin-1-ir neurons of mNTS (postremal level)	immunohistochemistry fasted rats	[60]
Immunological stress				
Lipopolysaccharide	ip injection lipopolysaccharide at a dose of 250 µg/kg, sacrificed 3 hours after injection	Fos induction in nesfatin-1-ir neurons of SON, PVN, Arc, NTS	immunohistochemistry fasted rats	[61]

Table 1. contd….

Stressor	Details of Stress Paradigm and Duration	Effects	Method	Refs.
Immunological stress				
Lipopolysaccharide	ip injection of LPS at a dose of 100µg/kg body weight, sacrificed at 2 hours	increased gastric corpus NUCB2 protein concentration at 2 h post injection	Western Blot ad libitum fed rats	[62]
Lipopolysaccharide	ip injection of LPS at a dose of 100µg/kg body weight, sacrificed at 2 hours	increased gastric corpus NUCB2 mRNA concentration at 2 h post injection	q-RT-PCR ad libitum fed rats	[62]
Lipopolysaccharide	ip injection of LPS at a dose of 100µg/kg body weight, venous blood withdrawal through iv catheter at 2, 5, 7, 24 hours	increased plasma NUCB2/nesfatin-1 at 2, 5 and 7 hours after injection	radioimmunoassay ad libitum fed rats	[62]
Combination stressor				
Chronic variable mild stress	14 days: swim stress, 2 min (4 °C); humid sawdust, 3 h food/water deprivation, permanent lights on, overnight; humid sawdust, permanent lights off, 180 min; swim stress, 2 min (4 °C) food/water deprivation, overnight; isolation, overnight cold isolation (4 °C), 15 min; lights off, 120 min swim stress, 4 min (12 °C); food/water deprivation, overnight inverted light/dark cycle; humid sawdust, overnight constant light, overnight; food/water deprivation, overnight lights off, 180 min; humid sawdust, permanent isolation, overnight; food/water deprivation, overnight restraint stress, 60 min; lights on, overnight inverted light/dark cycle; food/water deprivation, overnight Restraint stress, 60 min	Fos induction in CART/nesfatin-1-ir neurons of npEW increase in nesfatin-1 ir in npEW	Fos immunohistochemistry rats	[47]
Chronic variable mild stress	see protocol above	no effect on NUCB2 mRNA in npEW	qRT PCR rats	[47]
Metabolic stress				
Hypoglycemia	fasted rats ip injection with insulin (Actrapid, 10 U/kg)	Fos induction in nesfatin-1-ir neurons of PVN, Arc, LHA, all levels of NTS, DMN	immunohistochemistry fasted rats	[63]
Hypoglycemia	ip injection (300 mg/kg) with 2-deoxyglucose (2-DG) solution or ICV injection with 2-DG solution (4 mg/rat, 12 µl, 2 µl/min)	Fos induction in nesfatin-1-ir neurons of PVN, Arc, LHA, all levels of NTS, DMN	immunohistochemistry fasted rats	[63]

Abbreviations: apPVN, anterior parvicellular part of the paraventricular nucleus; Arc, arcuate nucleus; BNST, bed nucleus of the stria terminalis; CART, cocaine and amphetamine regulated transcript; cNTS, caudal part of the nucleus of the solitary tract; DMN, dorsal motor nucleus of the vagus nerve; EW, Edinger-Westphal nucleus; icv, intracerebroventricular; ir, immunoreactive; ip, intraperitoneal; iv, intravenous; LHA, lateral hypothalamic area; LC, locus coeruleus; lmPVN, lateral magnocellular part of the paraventricular nucleus; LPS, lipopolysaccharide; mmPVN, medial magnocellular part of the paraventricular nucleus; mNTS, medial part of the nucleus of the solitary tract; mpPVN, medial parvicellular part of the paraventricular nucleus; npEW, nonpreganglionic Edinger-Westphal nucleus; NTS, nucleus of the solitary tract; PVN, paraventricular nucleus; rNTS, rostral part of the nucleus of the solitary tract; rRPA, rostral raphe pallidus; SON, supraoptic nucleus; VLM, ventrolateral medulla.

expression [62] pointing towards a gastric source of circulating NUCB2/nesfatin-1. These data show that central nesfatin-1 positive neurons are activated and gastric NUCB2 production and release are increased in response to peripheral LPS injection leading to the hypothesis that activation of nesfatin-1 signalling could underlie the reduced food intake and decreased gastric emptying observed under conditions of peripheral inflammation.

Combination Stressor

Chronic Variable Mild Stress

In contrast to the growing body of evidence using acute stress models, only few chronic stress studies are available. Xu *et al*. used a combination of swimming, restraint, food deprivation, cold stress, light-dark inversion and isolation as a chronic variable mild stress paradigm (CVMS) [47]. This stressor induced Fos expression in nesfatin-1-containing neurons in the npEW [47]. However, there was no change in NUCB2 mRNA expression in the EW [47]. A follow up study confirmed the lack of NUCB2 mRNA changes also in the hypothalamus, a finding in line with the absence of changes in NUCB2/nesfatin-1 plasma levels [54]. Whether these data indicate that NUCB2/nesfatin-1 is not responsive to subacute/repeated stress or whether compensatory and/or adaptive mechanisms were already recruited warrants further investigation.

Metabolic Stress

Hypo- and Hyperglycemia

Peripheral insulin-induced hypoglycemia (by ip injection) or intracellular glucopenia caused by ip or icv injection of the glucose antimetabolite 2-deoxyglucose activate NUCB2/nesfatin-1 positive neurons in the arcuate nucleus, PVN, LHA, NTS and most prominently in the dorsal motor nucleus of the vagus nerve (DMN) [63]. Retrograde labelling studies showed that preganglionic NUCB2/nesfatin-1 positive neurons of the DMN that were activated by hypoglycemia target the stomach and the pancreas [63], which implies that central nesfatin-1 takes part in the regulation of glucose homeostasis. This hypothesis has been corroborated by a study showing that third ventricular injection of nesfatin-1 improves peripheral and hepatic insulin sensitivity in rats fed a high fat diet [64].

Also hyperglycemia alters NUCB2/nesfatin-1 signalling with decreased hypothalamic NUCB2 mRNA levels observed in hyperglycemic TsumuraSuzuki Obese Diabetes mice, a finding that might contribute to the hyperphagia observed in these mice [65]. Interestingly, pancreatic islet NUCB2 mRNA expression in humans is down-regulated under conditions of type 2 diabetes mellitus as well [66], likely contributing to the lower NUCB2/nesfatin-1 plasma levels observed [67]. One might speculate that reduced NUCB2/nesfatin-1 signalling under these conditions is deleterious and further aggravates the disease, a finding indirectly supported by a recent study showing an association between reduced circulating NUCB2/nesfatin-1 levels and peripheral arterial disease in patients with type 2 diabetes mellitus [68]. Whether a pharmacological increase of nesfatin-1 levels will help to prevent or reverse these alterations will have to be tested in future studies.

Exercise

The effect of acute metabolic stress was investigated in male kickboxers that were subjected to acute strenuous interval exercise and circuit exercise. Although this stress paradigm had effects on growth hormone, insulin or glucose, nesfatin-1 levels were not changed [69]. The lack of changes of circulating NUCB2/nesfatin-1 was confirmed in another study applying exercise with maximal fat oxidation intensity, while exercise at the individual anaerobic threshold led to a reduction of plasma NUCB2/nesfatin-1 in overweight men [70]. Using a repeated exercise protocol, another group recently reported that repeated high-intensity interval training in overweight men (three days per week over a period of six weeks) resulted in an increase of circulating NUCB2/nesfatin-1 levels compared to controls, while moderate-intensity continuous exercise training did not [71]. This partly discrepant results – although these studies are difficult to compare due to different exercise protocols and time courses – should be followed up in future studies investigating the direction of NUCB2/nesfatin-1 alterations by using different well-defined exercise protocols under several time course conditions.

SUMMARY

NUCB2/nesfatin-1 has been early on well-established as an anorexigenic modulator of food intake. The recent years have witnessed an increase of evidence on the role of nesfatin-1 in several other homeostatic functions. One main function seems to be the involvement in the response to stress, an effect that may not only take place on the central but also peripheral level. This finding has been shown under very different conditions using different stress models, ranging from psychological, to physical, immunological and metabolic stressors and is likely to contribute to the mainly inhibitory gastrointestinal effects observed. Future studies should further investigate the mechanisms underlying these effects and test whether these findings can also be translated to humans.

KEY POINTS

- NUCB2/nesfatin-1 is a well-established inhibitor of food intake.
- NUCB2/nesfatin-1 also plays an important modulatory role in the stress response.
- An activation of central (and also peripheral) NUCB2/nesfatin-1 signalling has been shown following various stressors (psychological, physical, immunological and metabolic).
- This activation likely contributes to the mainly inhibitory effects of upper gastrointestinal functions.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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